REMARKS

Upon entry of the instant Preliminary Amendment, the Applicants believe the application will be in condition for allowance and early notification thereof is solicited. The Commissioner is hereby authorized to charge any additional fees which may be required in the Application to Deposit Account No. 54-1214.

Respectfully submitted,

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APPENDIX TO THE IN THE SPECIFICATION:

Replace the paragraph starting at page 1, lines 7-12, with the following rewritten paragraph:

- - This **application is a divisional of U.S. Application No. 07/941,762, filed September 4, 1992; which is a continuation of U.S. Application No. 07/809,083, filed December 9, 1991; which is a continuation of U.S. Application No. 07/446,332, filed December 4, 1989,** which is a continuation-in-part application of copending application Serial Number 353,235 having the same title and filed May 16, 1989, and Serial Number 353,241, **now abandoned,** having the same title and filed May 17, 1989, the disclosures of which are hereby incorporated by reference.- -

Replace the paragraph bridging pages 6 and 7 with the following rewritten paragraph:

- - Figure 3 Amino acid sequence of the V_H regions of 19 mouse monoclonal antibodies with specificity for phosphorylcholine **(SEQ ID NOS:1-19)**. The designation HP indicates that the protein is the product of a hybridoma. The remainder are myeloma proteins. (From Gearhart et al., Nature, 291:29, 1981.) - -

Please replace the paragraph starting at page 7, lines 15-19, with the following rewritten paragraph:

- - Figure 5 Nucleotide sequences are clones from the cDNA library of the PCR amplified V_H regions in Lambda ZAP. The N-terminal 110 bases are listed here and the underlined nucleotides represent CDR1 (complementary determining region)**(SEQ ID NOS:20-37)**.- -

Replace the paragraph bridging pages 7 and 8 with the following rewritten paragraph:

- -Figure 6 The sequence of the synthetic DNA insert inserted into Lambda ZAP to produce Lambda Zap II V_H (Panel A **(SEQ ID NO:38)**) and Lambda Zap V_L (Panel B **(SEQ ID NO:39)**) expression vectors. The various features required for this vector to express the V_H and V_L-coding DNA homologs include the Shine-Dalgarno ribosome binding site, a leader sequence to direct the expressed protein to the periplasm as described by Mouva et al., J. Biol. Chem.,

255:27, 1980, and various restriction enzyme sites used to operatively link the V_H and V_L homologs to the expression vector. The V_H expression-vector sequence also contains a short nucleic acid sequence that codes for amino acids typically found in variable regions heavy chain (V_H Backbone). This V_H Backbone is just upstream and in the proper reading as the V_H DNA homologs that are operatively linked into the Xho I and Spe I. The V_L DNA homologs are operatively linked into the V_L sequence (Panel B) at the Nco I and Spe I restriction enzyme sites and thus the V_H Backbone region is deleted when the V_L DNA homologs are operatively linked into the V_L vector.- -

Replace the paragraph bridging pages 8 and 9 with the following rewritten paragraph:

--TGAATTCTAAACTAGTCGCCAAGGAGACAGTCATAATGAA
TCGAACTTAAGATTTGATCAGCGGTTCCTCTGTCAGTATTACTT
ATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTG
TATGGATAACGGATGCCGTCGGCGACCTAACAATAATGAGCGAC

CCCAACCAGCCATGGCCGAGCTCGTCAGTTCTAGAGTTAAGCGGCCG
GGGTTGGTCGGTACCGGCTCGAGCAGTCAAGATCTCAATTCGCCGGCAGCT **(SEQ ID NO:40)** - -

Please replace the paragraph starting at page 9, lines 17-21, with the following rewritten paragraph:

- - Figure 10 The sequence of the synthetic DNA segment inserted into Lambda Zap II to produce the lambda V_LII-expression vector **(SEQ ID NO:41)**. The various features and restriction endonuclease recognition sites are shown.- -

Please replace the paragraph starting at page 10, lines 18-34, with the following rewritten paragraph:

- - Figure 14 PCR amplification of Fd and kappa regions from the spleen mRNA of a mouse immunized with NPN is illustrated. Amplification was performed as described in Example 18 using RNA cDNA hybrids obtained by the reverse transcription of the mRNA with primer specific for amplification of light chain sequences (Table 2 **(SEQ ID NOS:60-84)**)or heavy chain sequences (Table 1 **(SEQ ID NOS:42-59)**). Lanes F1-F8 represent the product of

heavy chain amplification reactions with one of each of the eight 5' primers (primers 2-9, Table 1) and the unique 3' primer (primer 15, Table 2). Light chain (k) amplifications with the 5' primers (primers 3-6, and 12, respectively, Table 2) and the appropriate 3' primer (primer 13, Table 2) are shown in lanes F9-F13. A band of 700 bps is seen in all lanes indicating the successful amplification of Fd and k regions.- -

Please replace the paragraph starting at page 13, lines 19-23, with the following rewritten paragraph:

- - Figure 18 The sequence of the synthetic DNA insert inserted into Lambda Zap II V_H to produce the selectable V_H expression vector (panel A **(SEQ ID NO:119)**) and Lambda Zap II V_L II according to Example 17 to produce the selectable V_L expression vector (panel B **(SEQ ID NO:120)**).- -

Please replace the paragraph starting at page 13, lines 24-36, with the following rewritten paragraph:

- - Figure 19

(A) The major features of the selectable V_L expression vector are shown in panel A. The feature of the synthetic DNA sequence from Figure 18A **(SEQ ID NO:119)** is shown at the top along with the T_3 polymerase promoter from Lambda Zap II. The orientation of the insert in Lambda Zapp II is shown. The V_H DNA homologs are inserted into the Xho I and Spe I restriction enzyme sites. The V_H DNA homologs are inserted into the Xho I and Spe I site and the read through transcription produces the decapeptide epitope (tag) that is located just 3' of the cloning sites.- -

Please replace the paragraph bridging pages 13 and 14, with the following rewritten paragraph:

- - (B) The major features of the bacterial expression vector Lambda Zap II V_H (V_H-expression vector) are shown. **[the] The** synthetic DNA sequence from Figure 6 **(SEQ ID NO:38)** is shown at the top along with the T_3 polymerase promoter from Lambda Zap II. The orientation of the insert in Lambda Zapp II is shown. The V_H DNA homologs are inserted into the Xho I and Spe I restriction enzyme sites. The V_H DNA are inserted into the Xho I and Spe I

site and the read through transcription produces the decapeptide epitope (tag) that is located just 3' of the cloning sites.- -

Replace the paragraph bridging pages 16 and 17 with the following rewritten paragraph:

- - B. Methods

The present invention provides a novel method for tapping the immunological repertoire by isolating from V_H-coding and V_L-coding gene repertoires genes coding for an antibody receptor **[heterodimeric]** **<u>heterodimer</u>** capable of binding a preselected ligand. Generally, the method combines the following elements: - -

Replace the paragraph bridging pages 43 and 44 with the following rewritten paragraph:

- - 2. <u>Lambda Zap II V_H</u>

Lambda Zap II V_H is prepared by inserting the synthetic DNA sequences illustrated in Figure 6A **<u>(SEQ ID NO:38)</u>** into the above-described Lambda Zap II vector. The inserted nucleotide sequence advantageously provides a ribosome binding site (Shine-Dalgarno sequence) to permit proper imitation of mRNA translation into protein, and a leader sequence to efficiently direct the translated protein to the periplasm. The preparation of Lambda Zap II V_H is described in more detail in Example 9, and its features illustrated in Figures 6A and 7.- -

Please replace the paragraph starting at page 44, lines 2-7, with the following rewritten paragraph:

- - 3. <u>Lambda Zap II V_L</u>

Lambda Zap II V_L is prepared as described in Example 12 by inserting into Lambda Zap II the synthetic DNA sequence illustrated in Figure 6B. Important features of Lambda Zap II V_L are illustrated in Figure 8 **<u>(SEQ ID NO:39)</u>**. - -

Replace the paragraph bridging pages 44 and 45 with the following rewritten paragraph:

- - 1. <u>Polynucleotide Selection</u>

The nucleotide sequences encoding the immunoglobulin protein CDR's are highly variable. However, there are several regions of conserved sequences that flank the V_H domains. For instance, contain substantially conserved nucleotide sequences, i.e., sequences that will hybridize to the same primer sequence. Therefore, polynucleotide synthesis (amplification) primers that hybridize to the conserved sequences and incorporate restriction sites into the DNA

homolog produced that are suitable for operatively linking the synthesized DNA fragments to a vector were constructed. More specifically, the DNA homologs were inserted into Lambda ZAP II vector (Stratagene Cloning System, San Diego, CA) at the Xho I and EcoR I sites. For amplification of the V_H domains, the 3' primer (primer 12 **(SEQ ID NO:53)** in Table 1), was designed to be complementary to the mRNA in the J_H region. In all cases, the 5' primers (primers 1-10 **(SEQ ID NOS:42-51)**, Table 1) were chosen to be complementary to the first strand cDNA in the conserved N-terminus region (antisense strand). Initially amplification was performed with a mixture of 32 primers (primer 1 **(SEQ ID NO:84)**, Table 1) that were degenerate at five positions. Hybridoma mRNA could be amplified with mixed primers, but initial attempts to amplify mRNA from spleen yielded variable results. Therefore, several alternatives to amplification using the mixed 5' primers were compared. - -

Please replace the paragraph starting at page 45, lines 18-24, with the following rewritten paragraph:

- - The first alternative was to construct multiple unique primers, eight of which are shown in Table 1, corresponding to individual members of the mixed primer pool. The individual primers 2-9 of Table 1 **(SEQ ID NOS:42-49)** were constructed by incorporating either of the two possible nucleotides at three of the five degenerate positions.- -

Replace the paragraph bridging pages 45 and 46 with the following rewritten paragraph:

- - The second alternative was to construct a primer containing inosine (primer 10 **(SEQ ID NOS:50-51)**, Table 1) at four of the variable positions based on the published work of Takahashi, et al., Proc. Natl. Acad. Sci. (U.S.A.), 82:1931-1935, (1985) and Ohtsuka et al., J. Biol. Chem., 260:2605-2608, (1985). This primer has the advantage that it is not degenerate and, at the same time minimizes the negative effects of mismatches at the unconserved positions as discussed by Martin et al., Nuc. Acids Res., 13:8927 (1985). However, it was not known if the presence of inosine nucleotides would result in incorporation of unwanted sequences in the cloned V_H regions. Therefore, inosine was not included at the one position that remains in the amplified fragments after the cleavage of the restriction sites. As a result, inosine was not in the cloned insert.- -

Please replace the paragraph starting at page 46, lines 7-16, with the following rewritten paragraph:

- - Additional, V amplification primers including the unique 3' primer were designed to be complementary to a portion of the first constant region domain of the gamma 1 heavy chain mRNA (primers 15 and 16 **(SEQ ID NOS:57-58)**, Table 1). These primers will produce DNA homologs containing polynucleotides coding for amino acids from the V_H and the first constant region domains of the heavy chain. These DNA homologs can therefore be used to produce Fab fragments rather than an F_V. - -

Please replace the paragraph starting at page 46, lines 17-25, with the following rewritten paragraph:

- - As a control for amplification from spleen or hybridoma mRNA, a set of primers hybridizing to a highly conserved region within the constant region IgG, heavy chain gene were constructed. The 5' primer (primer 11 **(SEQ ID NO:52)**, Table 1) is complementary to the cDNA in the C_H2 region whereas the 3' primer (primer 13, Table 1 **(SEQ ID NO:55)**) is complementary to the mRNA in the C_H3 region. It is believed that no mismatches were present between these primers and their templates. - -

Replace the paragraph bridging pages 46 and 47 with the following rewritten paragraph:

- - The nucleotide sequences encoding the V_L CDRs are highly variable. However, there are several regions of conserved sequences that flank the V_L CDR domains including the J_L, V_L framework regions and V_L leader/promotor. Therefore, amplification primers that hybridize to the conserved sequences and incorporate restriction sites that allowing cloning the amplified fragments into the pBluescript SK-vector cut with Nco I and Spe I were constructed. For amplification of the V_L CDR domains, the 3' primer (primer 14 **(SEQ ID NO:56)** in Table 1), was designed to be complementary to the mRNA in the J_L regions. The 5' primer (primer 15 **(SEQ ID NO:57)**, Table 1) was chosen to be complementary to the first strand cDNA in the conserved N-terminus region (antisense strand). - -

Please replace the paragraph starting at page 47, lines 6-18, with the following rewritten paragraph:

- - A second set of amplification primers for amplification of the V_L CDR domains the 5' primers (primers 1-8 **(SEQ ID NOS:60-66 and SEQ ID NO:121)** in Table 2) were designed to be complementary to the first strand cDNA in the conserved N-terminus region. These primers also introduced a Sac I restriction endonuclease site to allow the V_LDNA homolog to be cloned into the V_LII-expression vector. The 3' V_L amplification primer (primer 9 **(SEQ ID NO:67)** in Table 2) was designed to be complementary to the mRNA in the J_L regions and to introduce the Xba I restriction endonuclease site required to insert the V_LDNA homolog into the V_LII-expression vector (Figure a). - -

Please replace the paragraph starting at page 47, lines 19-26, with the following rewritten paragraph:

- - Additional 3' V_L amplification primers were designed to hybridize to the constant region of either kappa or lambda mRNA (primers 10 and 11 **(SEQ ID NOS:68-69)** in Table 2). These primers allow a DNA homolog to be produced containing polynucleotide sequences coding for constant region amino acids of either kappa or lambda chain. These primers make it possible to produce an Fab fragment rather than an F_V. - -

Replace the paragraph bridging pages 47 and 48 with the following rewritten paragraph:

- - The primers used for amplification of kappa light chain sequences for construction of Fabs are shown at least in Table 2. Amplification with these primers was performed in 5 separate reactions, each containing one of the 5' primers (primers 3-6 **(SEQ ID NOS:62-65)**, and 12 **(SEQ ID NO:70)**) and one of the 3' primers (primer 13 **(SEQ ID NO:71)**). The remaining 3' primer (primer 9 **(SEQ ID NO:67)**) has been used to construct F_V fragments. The 5' primers contain a Sac I restriction site and the 3' primers contain a Xba I restriction site. - -

Please replace the paragraph starting at page 48, lines 2-15, with the following rewritten paragraph:

- - The primers used for amplification of heavy chain Fd fragments for construction of Fabs are shown at least in Table 1. Amplification was performed in eight separate reactions, each containing one of the 5' primers (primers 2-9 **(SEQ ID NOS:42-49)**) and one of the 3' primers (primer 15 **(SEQ ID NO:57)**). The remaining 5' primers that have been used for amplification in a single reaction are either a degenerate primer (primer 1 **(SEQ ID NO:84)**) or a

primer that incorporates inosine at four degenerate positions (primer 10 **(SEQ ID NOS:50-51)**, Table 1, and primers 17 and 18, Table 2 **(SEQ ID NOS:75-76)**). The remaining 3' primer (primer 14 **(SEQ ID NO:72)**, Table 2) has been used to construct F_V fragments. Many of the 5' primers incorporate a Xho I site, and the 3' primers include a Spe I restriction site. - -

Replace Tables 1 and 2 on pages 49-51 with the following Tables:

TABLE 1

(1)	5' AGGT[(C/G)(C/A)]**SM**A[(G/A)]**R**CT[(G/T)]**K**CTCGAGTC[(T/A)]**W**GG 3' **(SEQ ID NO:84)**	degenerate 5' primer for the amplification of variable heavy chain region (V_H)
(2)	5' AGGTCCAGCTGCTCGAGTCTGG 3' **(SEQ ID NO:42)**	Unique 5' primer for the amplification of V_H
(3)	5' AGGTCCAGCTGCTCGAGTCAGG 3' **(SEQ ID NO:43)**	"
(4)	5' AGGTCCAGCTTCTCGAGTCTGG 3' **(SEQ ID NO:44)**	"
(5)	5' AGGTCCAGCTTCTCGAGTCAGG 3' **(SEQ ID NO:45)**	"
(6)	5' AGGTCCAACTGCTCGAGTCTGG 3' **(SEQ ID NO:46)**	"
(7)	5' AGGTCCAACTGCTCGAGTCAGG 3' **(SEQ ID NO:47)**	"
(8)	5' AGGTCCAACTTCTCGAGTCTGG 3' **(SEQ ID NO:48)**	"
(9)	5' AGGTCCAACTTCTCGAGTCAGG 3' **(SEQ ID NO:49)**	"
(10)	5' AGGTIIAICTICTCGAGTC**[T/A]T**) 3' **(SEQ ID NO:50)**	5' degenerate primer containing inosine at 4 degenerate positions
(10)	**5' AGGTIIAICTICTCGAGTC(A) 3' (SEQ ID NO:51)**	**5' degenerate primer containing inosine at 4 degenerate positions**
(11)	5' GCCCAAGGATGTGCTCACC 3' **(SEQ ID NO:52)**	5' primer for amplification in the C_H2 region of mouse IgG1
(12)	5' CTATTAGAATTCAACGGTAACAGTGGTGCCTTGGCCCCA 3' **(SEQ ID NO:53)**	3' primer for amplification of V_H
(12A)	5' CTATTAACTAGTAACGGTAACAGTGGTGCCTTGGCCCCA 3' **(SEQ ID NO:54)**	3' primer for amplification of V_H using 3' Spe I site
(13)	5' CTCAGTATGGTGGTTGTGC 3' **(SEQ ID NO:55)**	3' primer for amplification in the C_H3 region of mouse IgG1
(14)	5' GCTACTAGTTTTGATTTCCACCTTGG 3' **(SEQ ID NO:56)**	3' primer for amplification of V_L
(15)	5' CAGCCATGGCCGACATCCAGATG 3' **(SEQ ID NO:57)**	5' primer for amplification of VL
(16)	5' AATTTTACTAGTCACCTTGGTGCTGCTGGC 3' **(SEQ ID NO:58)**	Unique 3' primer for amplification of V_H including part of the mouse gamma 1 first constant
(17)	5' TATGCAACTAGTACAACCACAATCCCTGGGCACAATTTT 3' **(SEQ ID NO:59)**	Unique 3' primer for amplification of V_H including part of mouse gamma 1 first constant region and hinge region

TABLE 2

(1)	5' CCAGTTCCGAGCTCGTTGTGACTCAGGAATCT 3' **(SEQ ID NO:60)**	Unique 5' primer for the amplification of V_L
(2)	5' CCAGTTCCGAGCTCGTGTTGACGCAGCCGCCC 3' **(SEQ ID NO:61)**	" "
(3)	5' CCAGTTCCGAGCTCGTGCTCACCCAGTCTCCA 3' **(SEQ ID NO:62)**	" "
(4)	5' CCAGTTCCGAGCTCCAGATGACCCAGTCTCCA 3' **(SEQ ID NO:63)**	" "
(5)	5' CCAGATGTGAGCTCGTGATGACCCAGACTCCA 3' **(SEQ ID NO:64)**	" "
(6)	5' CCAGATGTGAGCTCGTCATGACCCAGTCTCCA 3' **(SEQ ID NO:65)**	" "
(7)	5' CCAGATGTGAGCTCTTGATGACCCAAACTCAA 3' **(SEQ ID NO:66)**	" "
(8)	5' CCAGATGTGAGCTCGTGATAACCCAGGATGAA 3' **(SEQ ID NO:121)**	" "
(9)	5' GCAGCATTCTAGAGTTTCAGCTCCAGCTTGCC 3' **(SEQ ID NO:67)**	Unique 3' primer for V_L amplification
(10)	5' CCGCCGTCTAGAACACTCATTCCTGTTGAAGCT 3' **(SEQ ID NO:68)**	Unique 3' primer for V_L amplification including the kappa constant region
(11)	5' CCGCCGTCTAGAACATTCTGCAGGAGACAGACT 3' **(SEQ ID NO:69)**	Unique 3' primer for V_L amplification including the lambda constant region
(12)	5' CCAGTTCCGAGCTCGTGATGACACAGTCTCCA 3' **(SEQ ID NO:70)**	Unique 5' primer for V_L amplification
(13)	5' GCGCCGTCTAGAATTAACACTCATTCCTGTTGAA 3' **(SEQ ID NO:71)**	Unique 3' primer for V_L amplification
(14)	5' CTATTAACTAGTAACGGTAACAGTGGTGCCTTGCCCCA 3' **(SEQ ID NO:72)**	Unique 3' primer for V_L amplification

TABLE 2 (Continued)

(15)	5'	AGGCTTACTAGTACAATCCCTGGGCACAAT 3' **(SEQ ID NO:73)**	Unique 3' primer for V_H amplification
(16)	5'	GCCGCTCTAGAACACTCATTCCTGTTGAA 3' **(SEQ ID NO:74)**	Unique 3' primer for V_L amplification
(17)	5'	AGGTIIAICTICTCGAGTCTGC 3' **(SEQ ID NO:75)**	Degenerate 5' primer containing inosine at 4 degenerate positions
(18)	5'	AGGTIIAICTICTCGAGTCAGC 3' **(SEQ ID NO:76)**	Degenerate 5' primer containing inosine at 4 degenerate positions
(19)	5'	GTGCCAGATGTGAGCTCGTGATGACCCAGTCTCCA 3' **(SEQ ID NO:77)**	Unique 5' primer for human kappa V_L amplification
(20)	5'	TCCTTCTAGATTACTAACACTCTCCCCTGTTGAA 3' **(SEQ ID NO:78)**	Unique 3' primer for human kappa V_L amplification
(21)	5'	GCATTCTAGACTATTAACATTCTGTAGGGGC 3' **(SEQ ID NO:79)**	Unique 3' primer for human lambda V_L amplification
(22)	5'	CTGCACAGGGTCCTGGGCCGAGCTCGTGGTGACTCAG 3' **(SEQ ID NO:80)**	Unique 5' primer for human lambda V_L amplification
(23)	5'	AGITGCAIITGCTCGAGTCTGG 3' **(SEQ ID NO:81)**	5' degenerate primer for human V_H amplification containing inosine at 3 degenerate positions
(24)	5'	GTGGGCATGTGTGAGTTGTGTCACTAGTTGGGGTTTTGAGCTC 3' **(SEQ ID NO:82)**	Unique 3' primer for human V_H amplification
(25)	5'	CGGACTAGTACAAGATTTGGGCTCTGCTTT 3' **(SEQ ID NO:83)**	Unique 3' primer for human IgG1 V_H amplification

Please replace the paragraph starting at page 56, lines 1-15, with the following rewritten paragraph:

- - 5. DNA Homolog Preparation

In preparation for PCR amplification, mRNA prepared according to the above examples was used as a template for cDNA synthesis by a primer extension reaction. In a typical 50 ul transcription reaction, 5-10 ug of spleen or hybridoma mRNA in water was first hybridized (annealed) with 500 ng (50.0 pmol) of the 3' V_H primer (primer 12 **(SEQ ID NO:53)**, Table 1) at **[65C]** **65° C** for five minutes. Subsequently, the mixture was adjusted to 1.5 mM dATP, dCTP, dGTP and dTTP, 40 mM Tris-HCl at pH 8.0, 8 mM $MgCl_2$, 50 mM NaCl, and 2mM spermidine. Moloney-Murine Leukemia virus Reverse transcriptase (Stratagene Cloning Systems), 26 units, was added and the solution was maintained for 1 hour at **[37C]** **37° C**. - -

Please replace the paragraph starting at page 56, lines 16-28, with the following rewritten paragraph:

- -PCR amplification was performed in a 100 ul reaction containing the products of the reverse transcription reaction (approximately 5 ug of the cDNA/RNA hybrid), 300 ng of 3' V_H primer (primer 12 **(SEQ ID NO:53)** of Table 1), 300 ng each of the 5' V_H primers (primer 2-10 **(SEQ ID NOS:42-51)** of Table 1) 200 mM of a mixture of dNTP's, 50 mM KC1, 10 mM Tris-HCl pH 8.3, 15 mM $MgCl_2$, 0.1% gelatin and 2 units of Taq DNA polymerase. The reaction mixture was overlaid with mineral oil and subjected to 40 cycles of amplification. Each amplification cycle involved denaturation at **[92C]** **92° C** for 1 minute, annealing at **[52C]** **52° C** for 2 minutes and polynucleotide synthesis by Primer extension (elongation) at **[72C]** **72° C** for 1.5 minutes. The amplified V_H-coding DNA homolog containing samples were extracted twice with phenol/chloroform, once with chloroform, ethanol precipitated and were stored at **[-70C]** **-70° C** in 10 mM Tris-HCl, (pH, 7.5) and 1 mM EDTA.- -

Replace the paragraph bridging pages 56 and 57 with the following rewritten paragraph:

- - Using unique 5' primers (2-9 **(SEQ ID NOS:42-49)**, Table 1), efficient V_H-coding DNA homolog synthesis and amplification from the spleen mRNA was achieved as shown in Figure 3, lanes R17-R24. The amplified cDNA (V_H-coding DNA homolog) is seen as a major band of the expected size (360 bp). The intensities of the amplified V_H-coding polynucleotide

fragment in each reaction appear to be similar, indicating that all of these primers are about equally efficient in initiating amplification. The yield and quality of the amplification with these primers was reproducible.- -

Please replace the paragraph starting at page 57, lines 11-32, with the following rewritten paragraph:

- -The primer containing inosine also synthesized amplified V_H-coding DNA homologs from spleen mRNA reproducibly, leading to the production of the expected sized fragment, of an intensity similar to that of the other amplified cDNAs (Figure 4, Lane R16). This result indicated that the presence of inosine also permits efficient DNA homolog synthesis and amplification. Clearly indicating how useful such primers are in generating a plurality of V_H-coding **[Dna]** **<u>DNA</u>** homologs. Amplification products obtained from the constant region primers (primers 11 and 13 **<u>(SEQ ID NOS:52 & 55)</u>**, Table 1) were more intense indicating that amplification was more efficient, possibly because of a higher degree of homology between the template and primers (Figure 4, Lane R9). Based on these results, a V_H-coding gene library was constructed from the products of eight amplifications, each performed with a different 5' primer. Equal portions of the products from each primer extension reaction were mixed and the mixed product was then used to generate a library of V_H-coding DNA homolog-containing vectors.- -

Replace the paragraph bridging pages 57 and 58 with the following rewritten paragraph:

- - DNA homologs of the V_H were prepared from the purified mRNA prepared as described above. In preparation for PCR amplification, mRNA prepared according to the above examples was used as a template for cDNA synthesis. In a typical 50 ul transcription reaction, 5-10 ug of spleen or hybridoma mRNA in water was fired annealed with 300 ng (50.0 pmol) of the 3' V_L primer (primer 14 **<u>(SEQ ID NO:56)</u>**, Table 1), at **[65C]** **<u>65° C</u>** for five minutes. Subsequently, the mixture was adjusted to 1.5 mM dATP, dCTP, dGTP, and dTTP, 40 mM Tris-HCl at pH 8.0, 8 mM $MgCl_2$, 50 mM NaCl, and 2 mM spermidine. Moloney-Murine Leukemia virus reverse transcriptase (Stratagene Cloning Systems), 26 units, was added and the solution was maintained for 1 hour at **[37C]** **<u>37° C</u>**. The PCR amplification was performed in 100 ul reaction containing approximately 5 ug of the cDNA/RNA hybrid produced as described above,

300 ng of the 3' V_L primer (primer 14 **(SEQ ID NO:56)** of Table 1), 300 ng of the 5' V_L primer (primer 15 **(SEQ ID NO:57)** of Table 1), 200 mM of a mixture of dNTP's, 50 mM KC1, 10 mM Tris-HCl pH 8.3, 15 mM $MgCl_2$, 0.1% gelatin and 2 units of Taq DNA polymerase. The reaction mixture was overlaid with mineral oil and subjected to 40 cycles of amplification. Each amplification cycle involved denaturation at **[92C]** 92° C for 1 minute, annealing at **[52C]** **52° C** for 2 minutes and elongation at **[72C]** **72° C** for 1.5 minutes. The amplified samples were extracted twice with phenol/chloroform, once with choloroform, ethanol precipitated and were stored at **[-70C]** **-70 ° C** in 10 mM Tris-HCl at 7.5 and 1 mM EDTA. - -

Replace the paragraph bridging pages 62 and 63 with the following rewritten paragraph:

- - The diversity of the population was assessed by classifying the sequenced clones into predefined subgroups (Figure 5 **(SEQ ID NOS:20-37)**). Mouse V_H sequences are classified into eleven subgroups (Figure 5). Mouse V_H sequences are classified into eleven subgroups [I (A,B,), II (A,B,C), III (A,B,C,D,) V (A, B)] based on framework amino acid sequences described in Sequences of Proteins of Immunological Interest by Kabot et al., 4th ed., U.S. Dept. of Health and Human Sciences, (1987); Dildrop, Immunology Today, 5:84, (1984); and Brodeur et al., Eur. J. Immunol., 14; 922, (1984). Classification of the sequenced clones demonstrated that the cDNA library contained V_H sequences of at least 7 different subgroups. Further, a pairwise comparison of the homology between the sequenced clones showed that no two sequences were identical at all positions, suggesting that the population is diverse to the extent that it is possible to characterize by sequence analysis. - -

Please replace the paragraph starting at page 63, lines 6-12, with the following rewritten paragraph:

- - Six of the clones **(L 36-50 (SEQ ID NOS:27; 24; 34; 20, 35, 37, 31)**, Figure 5) belong to the subclass III B and had very similar nucleotide sequences. This may reflect a preponderance of mRNA derived from one or several related variable genes in stimulated spleen, but the data does not permit ruling out the possibility of a bias in the amplification process. - -

Replace the paragraph bridging pages 63 and 64 with the following rewritten paragraph:

- - To express the plurality of V_H-coding DNA homologs in an E. coli host cell, a vector was constructed that placed the V_H-coding DNA homologs in the proper reading frame, provided

a ribosome binding site as described by Shine et al., Nature, 254:34, 1975, provided a leader sequence directing the expressed protein to the periplasmic space, provided a polynucleotide sequence that coded for a known epitope (epitope tag) and also provided a polynucleotide that coded for a spacer protein between the V_H-coding DNA homolog and the polynucleotide coding for the epitope tag. A synthetic DNA sequence containing all of the above polynucleotides and features was constructed by designing single stranded polynucleotide segments of 20-40 bases that would hybridize to each other and form the double stranded synthetic DNA sequence shown in Figure 6. The individual single-stranded polynucleotides (N_1-N_{12}) are shown in Table 3 **(SEQ ID NOS:85-96)**. - -

Replace the paragraph bridging pages 64 and 65 with the following rewritten paragraph:

- - Polynucleotides 2, 3, 9-4', 11, 10-5', 6, 7 and 8 were kinased by adding 1 μl of each polynucleotide (0.1 ug/ul) and 20 units of T_4 polynucleotide kinase to a solution containing 70 mM Tris-HCl at pH 7.6, 10 mM $MgCl_2$, 5 mM DTT, 10 mM 2ME, 500 micrograms per ml of BSA. The solution was maintained at **[37C]** **37° C** for 30 minutes and the reaction stopped by maintaining the solution at **[65C]** **65° C** for 10 minutes. The two end polynucleotides 20 ng of polynucleotides N1 **(SEQ ID NO:85)** and polynucleotides N12 **(SEQ ID NO:95)**, were added to the above kinasing reaction solution together with 1/10 volume of a solution containing 20.0 mM Tris-HCl at pH 7.4, 2.0 mM $MgCl_2$ and 50.0 MM NaCl. This solution was heated to **[70C]** **70° C** for 5 minutes and allowed to cool to room temperature, approximately **[25C]** **25° C**, over 1.5 hours in a 500 ml beaker of water. During this time period all 10 polynucleotides annealed to form the double stranded synthetic DNA insert shown in Figure 6A **(SEQ ID NO:38)**. The individual polynucleotides were covalently linked to each other to stabilize the synthetic DNA insert by adding 40 μl of the above reaction to a solution containing 50 mM Tris-HCl at pH 7.5, 7 mM $MgCl_2$, 1 mM DTT, 1 mM adenosine triphosphate (ATP) and 10 units of T4 DNA ligase. This solution was maintained at **[37C]** **37° C** for 30 minutes and then the T4 DNA ligase was inactivated by maintaining the solution at **[65C]** **65° C** for 10 minutes. The end polynucleotides were kinased by mixing 52 μl of the above reaction, 4 μl of a solution containing 10 mM ATP and 5 units of T4 polynucleotide kinase. This solution was maintained at **[37C]** **37° C** for 30 minutes and then the T4 polynucleotide kinase was inactivated by maintaining the solution at **[65C]** **65° C** for 10 minutes. The completed synthetic DNA insert was ligated directly into a

lambda Zap II vector that had been previously digested with the restriction enzymes Not I and Xho I. The ligation mixture was packaged according to the manufacture's instructions using Gigapack II Gold packing extract available from Stratagene Cloning Systems, La Jolla, CA. The packaged ligation mixture was plated on XL1 blue cells (Stratagene Cloning Systems, San Diego, CA). Individual lambda Zap II plaques were cored and the inserts excised according to the in vivo excision protocol provided by the manufacturer, Stratagene Cloning Systems, La Jolla, CA. This in vivo excision protocol moves the cloned insert from the lambda Zap II vector into a plasmid vector to allow easy manipulation and sequencing. The accuracy of the above cloning steps was confirmed by sequencing the insert using the Sanger dideoxide method described in by Sanger et al., Proc. Natl. Acad. Sri USA, 74:5463-5467, (1977) and using the manufacture's instructions in the AMV Reverse Transcriptase ^{35}S-ATP sequencing kit from Stratagene Cloning Systems, La Jolla, CA.- -

Please replace the paragraph starting at page 66, lines 1-2, with the following rewritten paragraph:

- - The sequence of the resulting V_H expression vector is shown in Figure 6A **(SEQ ID NO:38)** and Figure 7. - -

Please replace Table 3 starting at page 66, lines 4-17, with the following rewritten Table 3: - -

Table 3

Nl)	5'	GGCCGCAAATTCTATTTCAAGGAGACAGTCAT 3' **(SEQ ID NO:85)**
N2)	5'	AATGAAATACCTATTGCCTACGGCAGCCGCTGGATT 3' **(SEQ ID NO:86)**
N3)	5'	GTTATTACTCGCTGCCCAACCAGCCATGGCCC 3' **(SEQ ID NO:87)**
N4)	5'	AGGTGAAACTGCTCGAGAATTCTAGACTAGGTTAATAG 3' **(SEQ ID NO:88)**
N5)	5'	TCGACTATTAACTAGTCTAGAATTCTCGAG 3' **(SEQ ID NO:89)**
N6)	5'	CAGTTTCACCTGGGCCATGGCTGGTTGGG 3' **(SEQ ID NO:90)**
N7)	5'	CAGCGAGTAATAACAATCCAGCGGCTGCCGTAGGCAATAG 3' **(SEQ ID NO:91)**
N8)	5'	GTATTTCATTATGACTGTCTCCTTGAAATAGAATTTGC 3' **(SEQ ID NO:92)**
N9-4)	5'	AGGTGAAACTGCTCGAGATTTCTAGACTAGTTACCCGTAC 3' **(SEQ ID NO:93)**
N11)	5'	GACGTTCCGGACTACGGTTCTTAATAGAATTCG 3' **(SEQ ID NO:94)**
N12)	5'	TCGACGAATTCTATTAAGAACCGTAGTC 3' **(SEQ ID NO:95)**
N10-5)	5'	CGGAACGTCGTACGGGTAACTAGTCTAGAAATCTCGAG 3' **(SEQ ID NO:96)**

- -

Please replace the paragraph starting at page 66, lines 19-34 with the following rewritten paragraph:

-- 10. V_L Expression Vector Construction

To express the plurality of V_L coding polynucleotides in an E. coli host cell, a vector was constructed that placed the V_L coding polynucleotide in the proper reading frame, provided a ribosome binding site as described by Shine et al., Nature, 254:34, (1975), provided a leader sequence directing the expressed protein to the periplasmic space and also provided a polynucleotide that coded for a spacer protein between the V_L polynucleotide and the polynucleotide coding for the epitope tag. A synthetic DNA sequence containing all of the above polynucleotides and features was constructed by designing single stranded polynucleotide segments of 20-40 bases that would hybridize to each other and form the double stranded

synthetic DNA sequence shown in Figure 6B **(SEQ ID NO:39)**. The individual single-stranded polynucleotides (N_1-N_8) are shown in Table 3 **(SEQ ID NO:85-92)**. - -

Replace the paragraph bridging pages 67 and 68 with the following rewritten paragraph:

- - Polynucleotides N2 **(SEQ ID NO:86)**, N3 **(SEQ ID NO:87)**, N4 **(SEQ ID NO:88)**, N6 **(SEQ ID NO:90)**, N7 **(SEQ ID NO:91)** and N8 **(SEQ ID NO:92)** were kinased by adding 1 µl of each polynucleotide and 20 units of T_4 polynucleotide kinase to a solution containing 70 mM Tris-HCl at pH 7.6, 10 mM $MgCl_2$, 5 mM DDT, 10 mM 2ME, 500 micrograms per ml of BSA. The solution was maintained at **[37C]** 37° C for 30 minutes and the reaction stopped by maintaining the solution at **[65C]** 65° C for 10 minutes. The two end polynucleotides 20 ng of polynucleotides N1 **(SEQ ID NO:85)** and polynucleotides N5 **(SEQ ID NO:89)** were added to the above kinasing reaction solution together with 1/10 volume of a solution containing 20.0 mM Tris-HCl at pH 7.4, 2.0 mM $MgCl_2$ and 50.0 mM NaCl. This solution was heated to **[70 C]** 70° C for 5 minutes and allowed to cool to room temperature, approximately **[25C]** 25° C, over 1.5 hours in a 500 ml beaker of water. During this time period all the polynucleotides annealed to form the double stranded synthetic DNA insert. The individual polynucleotides were covalently linked to each other to stabilize the synthetic DNA insert with adding 40 µl of the above reaction to a solution containing 50 µl Tris-HCl at pH 7.5, 7 mM $MgCl_2$, 1 mM DTT, 1 mM ATP and 10 units of T4 DNA ligase. This solution was maintained at 37C for 30 minutes and then the T4 DNA ligase was inactivated by maintaining the solution at **[65C]** 65° C for 10 minutes. The end polynucleotides were kinased by mixing 52 µl of the above reaction, 4 µl of a solution recontaining 10 mM ATP and 5 units of T4 polynucleotide kinase. This solution was maintained at **[37C]** 37° C for 30 minutes and then the T4 polynucleotide kinase was inactivated by maintaining the solution at **[65C]** 65° C for 10 minutes. The completed synthetic DNA insert was ligated directly into a lambda Zap II vector that had been previously digested with the restriction enzymes Not I and Xho I. The ligation mixture was packaged according to the manufacture's instructions using Gigapack II Gold packing extract available from Stratagene Cloning Systems, La Jolla, CA. The packaged ligation mixture was plated on XL1-Blue cells (Stratagene Cloning Systems, La Jolla, CA). Individual lambda Zap II plaques were cored and the inserts excised according to the in vivo excision protocol provided by the manufacturer,

Stratagene Cloning Systems, La Jolla, CA and described in Short et al., Nucleic Acids Res., 16:7583-7600, 1988. This in vivo excision protocol moves the cloned insert from the lambda Zap II vector into a phagemid vector to allow easy manipulation and sequencing and also produces the phagemid version of the V_L expression vectors. The accuracy of the above cloning steps was confirmed by sequencing the insert using the Sanger dideoxide method described by Sanger et al., Proc. Natl. Acad. Aci. USA, 74:5463-5467, (1977) and using the manufacturer's instructions in the AMV reverse transcriptase ^{35}S-dATP sequencing kit from Stratagene Cloning Systems, La Jolla, CA. The sequence of the resulting V_L expression vector is shown in Figure 6 and Figure 8. - -

Replace the paragraph bridging pages 68 and 69 with the following rewritten paragraph:

- - 11. V_LII-Expression Vector Construction

To express the plurality of V_L-coding DNA homologs in an E. coli host cell, a vector was constructed that placed the V_L-coding DNA homologs in the proper reading frame, provided a ribosome binding site as described by Shine et al., Nature, 254:34, 1975, provided the Pel B gene leader sequence that has been previously used to successfully secrete Fab fragments in E. coli by Lei et al., J. Bac., 169:4379 (1987) and Better et al., Science, 240:1041 (1988), and also provided a polynucleotide containing a restriction endonuclease site for cloning. A synthetic DNA sequence containing all of the above polynucleotides and features was constructed by designing single stranded polynucleotide segments of 20-60 bases that would hybridize to each other and form the double stranded synthetic DNA sequence shown in Figure 10 **(SEQ ID NO:41)**. The sequence of each individual single-stranded polynucleotides (O1-O8) within the double stranded synthetic DNA sequence is shown in Table 4 **(SEQ ID NOS:86; 87; 97; 98; 99; 100; 101; 102)**. - -

Please replace Table 4 starting at page 71, lines 1-10, with the following rewritten Table 4. - -

TABLE 4

01) 5' TGAATTCTAAACTAGTCGCCAAGGAGACAGTCAT 3' **(SEQ ID NO:97)**

02) 5' AATGAAATACCTATTGCCTACGGCAGCCGCTGGATT 3' **(SEQ ID NO:86)**

03) 5' GTTATTACTCGCTGCCCAACCAGCCATGGCC 3' **(SEQ ID NO:87)**

04) 5' GAGCTCGTCAGTTCTAGAGTTAAGCGGCCG 3' **(SEQ ID NO:98)**

05) 5' GTATTTCATTATGACTGTCTCCTTGGCGACTAGTTTAGAA-TTCAAGCT 3' **(SEQ ID NO:99)**

06) 5' CAGCGAGTAATAACAATCCAGCGGCTGCCGTAGGCAATAG 3' **(SEQ ID NO:100)**

07) 5' TGACGAGCTCGGCCATGGCTGGTTGGG 3' **(SEQ ID NO:101)**

08) 5' TCGACGGCCGCTTAACTCTAGAAC 3' **(SEQ ID NO:102)**

- -

Please replace the paragraph starting at page 71, lines 12-24 with the following rewritten paragraph:

- - 12. $V_H + V_L$ Library Construction

To prepare an expression library enriched in V_H sequences, DNA homologs enriched in V_H sequences were prepared according to Example 6 using the same set of 5' primers but with primer 12A (Table 1 **(SEQ ID NO:54)**) as the 3' primer. These homologs were then digested with the restriction enzymes Xho I and Spe I and purified on a 1% agarose gel using the standard electroelution technique described in Molecular Cloning A Laboratory Manual, Maniatis et al., eds., Cold Spring Harbor, New York, (1982). These prepared V_H DNA homologs were then directly inserted into the V_H expression vector that had been previously digested with Xho I and Spe I. - -

Please replace the paragraph starting at page 83, lines 3-27 with the following rewritten paragraph:

- - The dominant S gene from Lambda Zap Sam 5 was isolated using the polymerase chain reaction. Briefly, Lambda Zap Sam 5 DNA was isolated using the methods described in Molecular Cloning: A Laboratory Manual, Maniatis et al., eds., Cold Spring Harbor, New York (1982). Lambda Zap Sam 5 DNA, 0.1 ug, was admixed with a buffer containing 150 ng of primer RG15 (Table 5 **(SEQ ID NO:103)**) and 150 ng of primer RG16 (Table 5 **(SEQ ID NO:104)**), 0.25 mM each of dTTP, dCTP, dGTP, and dATP (dNTPs), 50 mM KCl, 10mM Tris-HCl at pH 8.3, 1.5 mM $MgCl_2$, and 0.15% sterile gelatin. The resulting solution was heated to 91C for five minutes and then placed in a 54C water bath for five minutes. 0.5 microliters of

Tag polymerase (Perkin Elmer-Cetus, Norwalk, CT) was added and the solution overlaid with a layer of mineral oil. - -

Replace the paragraph bridging pages 85 and 86 with the following replacement paragraph:

- - A synthetic DNA sequence containing the suppressor tRNA gene and polynucleotide sequence coding for decapeptide tag was constructed by designing single stranded polynucleotide segments of 20-40 bases that would hybridize to each other and form the double stranded synthetic DNA sequence shown in Figure 18A **(SEQ ID NO:119)**. The individual single-stranded polynucleotides are shown in Table 5 **(SEQ ID NOS:103-118)**.- -

Replace Table 5 from page 87, lines 1-19, with the following rewritten Table 5. - -

- -

TABLE 5

R615)	5'-AATAAGCTTGATCTATCAGTAATCGACC-3' **(SEQ ID NO:103)**
R616)	5'-ATTAGATCTGAATTCTGACGTCCGTTATCAG-3' **(SEQ ID NO:104)**
926)	5'GATCCGCTTCCCGATAAGGGAGCAGGCCAGTAAAAGCATTACCTGTGGTGGGGTTC-3' **(SEQ ID NO:105)**
927)	5'-CCGAGCGGCCAAAGGGAGCAGACTCTAAATCTGCCGTCATCGACTTCGAAG-3' **(SEQ ID NO:106)**
928)	5'-GTTCGAATCCTTCCCCCACCACCATCACTTTCAAAAGTCCGA-3' **(SEQ ID NO:107)**
929)	5'-CTAGTCGGACTTTTGAAAGTGATGGTGGTGGGGGAAGGATTCGAACCTTCGAAGTC-3' **(SEQ ID NO:108)**
930)	5'-GATGACGGCAGATTTAGAGTCTGCTCCCTRTTGGCCGCTCGGGAACCCCACC-3' **(SEQ ID NO:109)**
931)	5' -ACAGGTAATGCTTTTACTGGCCTGCTCCCTTATCGGGAAGCG-3' **(SEQ ID NO:110)**
970)	5'-TCGAGCGCC-3' **(SEQ ID NO:111)**
971)	5'-GATCGGCGC-3' **(SEQ ID NO:112)**
972)	5'-CTAGGGCCT-3' **(SEQ ID NO:113)**
973)	5'-CTAGAGGCC-3' **(SEQ ID NO:114)**
974)	5'-CGCCC-3' **(SEQ ID NO:115)**
978)	5'-GATCGGGCGAGCT-3' **(SEQ ID NO:116)**
AB23)	5'-CTAGTTACCCGTACGACGTTCCGGACTACGCTTCTTAATAG-3' **(SEQ ID NO:117)**
AB24)	5'-AATTCTATTAAGAAGCGTAGTCCGGAACGTCGACGGGTAA-3' **(SEQ ID NO:118)**

- -

Please replace the paragraph starting at page 88, lines 9-19 with the following rewritten paragraph:

- - The required polynucleotides were annealed to form the synthetic DNA sequence shown in Figure 18A **<u>(SEQ ID NO:119)</u>**. Briefly, the following solutions of polynucleotides were admixed to 1/10 volume of a solution containing 20.0 mM Tris HCl at pH 7.4, 2.0 mM Mg CL_2 and 50.0 mM NaCl: 5 ul of separate, 2.5 ug/ml solutions containing the kinased polynucleotides 926, 927, 928, 929, 930 and 930; 4 ul of separate, 2.0 ug/ml solutions containing the unkinased polynucleotide AB24 and the kinased polynucleotide AB23; 2 ul of separate, 1.0 ug/ml solutions containing the kinased polynucleotide 971, and the unkinased polynucleotide 970. - -

Replace the paragraph bridging pages 88 and 89 with the following rewritten paragraph:

- - This solution was heated to **[70C]** **<u>70° C</u>** for 5 minutes and allowed to cool to **[40C]** **<u>40° C</u>** over 1.5 hours in a 500 ml beaker of water. During this time period all 10 polynucleotides annealed to form the double stranded synthetic DNA insert shown in Figure 18A **<u>(SEQ ID NO:119)</u>** . The individual polynucleotides were covalently linked to each other to stabilize the synthetic DNA insert by admixing all of the above reaction (46.6 ul) to a solution containing 50 mM Tris-HCl at pH 7.5, 7 mM $MgCl_2$, 1 mM DDT, 1 mM adenosine triphosphate (ATP) and 10 units of T4 DNA ligase to form a ligation reaction admixture. This admixture was maintained at **[37C]** **<u>37° C</u>** for 1 hour and then the T4 DNA ligase was inactivated by maintaining the solution at **[65C]** **<u>65° C</u>** for 15 minutes. The end polynucleotides were kinased by admixing all of the above ligation reaction admixture reaction, 6 μl of a solution containing 10 mM ATP and 5 units of T4 polynucleotide kinase. This solution was maintained at **[37C]** **<u>37° C</u>** for 30 minutes and then the T4 polynucleotide kinase was inactivated by maintaining the solution at **[65C]** **<u>65° C</u>** for 10 minutes. The completed synthetic DNA insert (Figure18A **<u>(SEQ ID NO:119)</u>**) was ready for ligation to the V_H-Expression vector (Figure 7) that had been previously digested with the restriction endonucleases Xho I and Eco RI.- -

Please replace the paragraph starting at page 90, lines 24-30 with the following rewritten paragraph:

- -A synthetic DNA sequence containing the suppressor tRNA gene and polynucleotide sequence coding for decapeptide tag was constructed by designing single stranded polynucleotide segments of 20-40 bases that would hybridize to each other and form the double stranded synthetic DNA sequence shown in Figure 18B **(SEQ ID NO:120)**. The individual single-stranded polynucleotides are shown in Table 5 **(SEQ ID NOS:103-118)**. - -

Please replace the paragraph starting at page 91, lines 4-12 with the following rewritten paragraph:

- - The required polynucleotides were annealed to form the synthetic DNA sequence shown in Figure 18B **(SEQ ID NO:120)** . Briefly, the following solutions of polynucleotides were admixed to 1/10 volume of a solution containing 20.0 mM Tris HCl at pH 7.4, 2.0 mM Mg CL_2 and 50.0 mM NaCl: 5 ul of separate, 2.5 ug/ml solutions containing the kinased polynucleotides 926, 927, 928, 929, 930 and 931; 2 ul of separate, 2.0 ug/ml solutions containing the unkinased polynucleotides 974 and 973, and the kinased polynucleotides 972 and 975. - -

Please replace the paragraph starting at page 91, lines 13-34 with the following rewritten paragraph:

- - This solution was heated to **[70C]** **70° C** for 5 minutes and allowed to cool to 40C over 1.5 hours in a 500 ml beaker of water. During this time period all 10 polynucleotides annealed to form the double stranded synthetic DNA insert shown in Figure 18B **(SEQ ID NO:120)**. The individual polynucleotides were covalently linked to each other to stabilize the synthetic DNA insert by admixing all of the above reaction (42.2 ul) to a solution containing 50 mM Tris-HCl at pH 7.5, 7 mM $MgCl_2$, 1 mM DDT, 1 mM adenosine triphosphate (ATP) and 10 units of T4 DNA ligase to form a ligation reaction admixture. This admixture was maintained at 37C for 1 hour and then the T4 DNA ligase was inactivated by maintaining the solution at 65C for 15 minutes. The end polynucleotides were kinased by admixing all of the above ligation reaction admixture reaction, 6 μl of a solution containing 10 mM ATP and 5 units of T4 polynucleotide kinase. This solution was maintained at **[37C]** **37° C** for 30 minutes and then the T4 polynucleotide kinase was inactivated day maintaining the solution at **[65C]** **65° C** for 10 minutes, the completed synthetic DNA insert (Figure 18B **(SEQ ID NO:120)**) was ready for

ligation to the V_H-Expression vector (Figure 9) that had been previously digested with the restriction endonucleases Sac I and Xba I. - -